REMARKS

1. General Matters

1.1. Restriction/Election

The Examiner has considered our traversal of the restriction, and maintained the restriction, without making the restriction FINAL. We believe that making the restriction FINAL is a prerequisite to petitioning against the restriction.

The Examiner clarifies that the holding of a posteriori lack of unity is based on the Anopheles gambiae sequence Genbank EAA01224, which is discussed in our election under the alternative accession numbers ref|XP_321524.1 and Genbank AAC49462.1.

The Examiner agrees with us that with percentage identity calculated as required by P7, L2-7, the percentage identity of the aligned sequence is 81.45%.

We wish to take this opportunity to correct the record. In our election with traverse, page 2, we stated that claim 1 had been amended to recite at least 85% identity. However, claim 1 in fact continued to recite 80% identity, albeit with clarification of how percentage identity was calculated. Higher percentage identities were recited in new claims 56 (90%), 57 (95%), and 58 (98%) and other sequence constraints were recited in new claims 59-63.

We have now in fact amended claim 1 to require 85% identity, thereby distinguishing EAA01224, and hence the holding of a posteriori lack of unity must be reconsidered.

1.2. Specification

The Examiner asks that the specification be updated to identify this application as the national stage of PCT/EP2004/051280.

This information is already in the ADS filed January 11, 2006. This is sufficient to satisfy 37 CFR 1.78(a)(2)(iii), and see also 37 CFR 1.76(a)(5) last sentence.

1.3. Drawings

The Examiner states that on Fig. 1, no "shaded areas" are visible to show similar residues, per page 5, line 35, referred to as "semi-conserved" residues on page 7, lines 9-11. The non-conserved residues of page 7, lines 11-13 are the normal typed black characters.

The shading is in fact plainly apparent on Fig. 1 in a printout from the IFW -- it is a background of dots.

Nonetheless, to facilitate examination, a replacement Fig. 1 is submitted herewith. The only difference between it and the original figure 1 is that the shading is half-tone rather than a discrete dot pattern.

1.4. Objections to Claims

Claim 55 has been amended to depend from claim 1.

2. Written Description (OA p. 4-7)

Claims 1-3, 6, 9, 16, 19, 20, 22, 55, 59-63, 65 and 66 are rejected as allegedly failing to comply with the written description requirement. By implication, examined claims 56 (at least 90% identity to SEQ ID NO:2), 57 (at least 95% identity), 58 (at least 98% identity), 64 (identical), 67 (high stringency hybridization), 68 (very high stringency), 69 (at least 90% with SEQ IDN O:1), 70 (at least 95%), 71 (at least 98%) and 72 (identical) are deemed to satisfy WD.

The Examiner asserts on page 5 of the office action that the claims recite 12 genera, identified by the Examiner by the letters "a" through "l". These should not be confused with paragraphs "a" through" "g" of original claim 1. The genera may be characterized as follows:

Genus	Claim	Comment
a	1	encodes polypeptide with at least 80% identity to SEQ ID NO:2
b	2	dependent on 1, adds functional limitation
c	6	dependent on 1 (the examiner fails to indicate that by virtue of this dependency, the claim also requires at least 80% identity to SEQ ID NO:2); further requires hybridization to SEQ ID NO:1
d	9	C-terminal truncation of SEQ ID NO:2; such truncations effectively limited by requirement of 80% identity over length of SID 2; thus cannot omit more than 20% of SID 2
e	59	80% mutants which include replacements at non-conserved or semi-conserved positions; the examiner mistakenly says that these positions are not identified when claim 59 specifically refers to Figure 1.
f	60	80% mutants which include replacement suggested by another insect kinase
g-j	63(i)-(iv)	defined conservative substitutions
k	65	medium stringency hybridization
l	66	medium/high stringency hybridization

With respect to genera (a)-(j), please note that claim 1 now requires the polynucleotide encode a kinase having at least 85% identity with SEQ ID NO:2.

With respect to genera (e)-(j), we have now amended claims 61, 63 and 65 to recite that the polypeptide differs from SEQ ID NO:2, "if at all", solely by the indicated mutations. Thus, each of these genera now includes SEQ ID NO:1, which encodes SEQ ID NO:2.

With respect to genus (c), claim 6 now requires

hybridization under medium stringency conditions.

The Examiner's analysis relies heavily on Guo (2004), which makes predictions concerning the percentage of random single substitution mutants which would be expected to be active. We consider this to be a classic example of knocking down a straw man.

The specification is directed to a person skilled in the art, for whom random single substitution over the entire length of the molecule would be a last resort.

The specification plainly teaches that

(1) certain residue positions are conserved, semi-conserved, or, by implication if not so marked, non-conserved, among four insect multisubstrate deoxynucleoside kinases (Fig. 1 and page 5, line 33 to page 6, line 2);

(2) residues which are semi-conserved or non-conserved are preferentially mutated (page 7, lines 8-13);

(3) mutations tolerated in DM-dNK according to WO01/88106 or Munch-Petersen (2000), notably certain C-terminal truncations, are likely to be tolerated in SID 2 (page 7, lines 13-15; page 8, line 25-page 19, line 5);

(4) amino acid substitutions are preferably "conservative substitutions" (page 8, line 35) as defined by page 8, line 37 to page 9, line 12;

(5) the residues closest to substrates, as determined by crystal structure of DM-dNK, are identified by asterisks in Fig. 1 (page 5, lines 36-38) and by implication the skilled worker should expect that mutation of these residues is likely to affect activity; and

(6) the overall level of mutation should be limited, see page 7, lines 2-7.

We additionally assert that it is within ordinary skill in the art:

(7) to identity residues tolerant or intolerant of mutation by alanine-scanning mutagenesis; and

(8) to prepare combinatorial libraries in which several

selected residues are exhaustively randomly mutated.

Resort to the directed random mutation techniques of (8) does not constitute undue-experimentation because of the ease of preparing and screening such libraries. Moreover, the "experiment" is the screening of the library, not of the original library member, and is successful if any member has activity, cp. In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988)

We respectfully direct the Examiner's attention to the PTO's Written Description Training Materials (Revision 1; March 25, 2008). The WDTM distinguishes between percentage identity claims which contain activity limitations, and those without. Claim 1 requires that the polynucleotide encode a "multisubstrate deoxyribonucleoside kinase", which is an activity limitation.

2.1. In view of the less stringent WD standard applied to a % identity claim without an activity limitation, we have presented new claims 73-75, which fall in this more permissive category. The relevant WDTM discussion is Ex. 10, model claim 2, Ex. 11A, model claim 1, and Ex. 1 model claim 1.

Referring first to Example 10, model claim 2, this claim reads: "An isolated variant of a protein comprising the amino acid sequence shown in SEQ ID NO:3, wherein the variant comprises an amino acid sequence that is at least 95% identical to SEQ ID NO:3".

The analysis of model claim 2 stated that in view of the disclosure of SEQ ID NO:3, those skilled in the art could readily envision all of the amino acid sequences that are at least 95% identical to SEQ ID NO:3 (page 34).

Model claim 1 of Ex. 11A reads, "An isolated nucleic acid that encodes a polypeptide with at least 85% amino acid sequence identity to SEQ ID NO:2".

The WDTM analysis (pp. 37-38) comments: "The disclosure of SEQ ID NO:2 combined with the pre-existing knowledge in the art regarding the genetic code and its redundancies would have put one in possession of the genus of nucleic acids that encode SEQ ID NO:22. With the aid of a computer, one of skill in the art

could have identified **all** of the nucleic acids that encode a polypeptide with at least 85% identity with SEQ ID NO:2".

Thus, new claims 73-75 conclusively satisfy the WD requirements as interpreted by the PTO's WDTM.

2.2. As previously noted, claim 1 (and 65) recite a "multisubstrate deoxyribonucleoside kinase" and thus contain an activity limitation.

The relevant WDTM analysis is of Example 10, model claim 3, Example 11A, model claim 2, and Example 11B, model claim 2.

The analysis of WDTM Example 10 model claim 3 assumed that (1) there was "no teaching in the specification regarding which 5% of the structure can be varied while retaining the ability of the protein to catalyze the reaction A→B", and (2) "there is no art-recognized correlation between any structure (other than SEQ ID NO:3) and the activity of catalyzing A→B, based on which those of ordinary skill in the art could predict which amino acids can vary from SEQ ID NO:3 without losing catalytic activity". On these facts, it held lack of WD.

The analysis was elaborated upon with respect to model claim 2 of Examples 11A and 11B. In Ex. 11A, the PTO assumed that an "art-recognized structure function correlation" was not present, and in 11B, that it was. As a result it found WD satisfied in 11B but not in 11A.

In the analysis of Ex. 11A, the PTO began by assuming that there was no significant sequence identity between the recited polypeptide and any known polypeptide, or disclosure of any additional activity polypeptides in the specification (page 37). It conceded that the skilled worker was aware of "amino acid substitution exchange groups" and that substitutions within the exchange group would be expected to conserve the overall tertiary structure[1]. However, it declined to consider this knowledge sufficient to assure conservation of function (pp. 38-39). (We wish to note that in the studies which were used to <u>define</u> the

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[1] At least if % identity was at least 50%, see WDTM p. 38.

exchange groups, the standard was in fact conservation of function, not 3D structure, because 3D structure data was available for fewer protein families.)

In Ex. 11B, the PTO assumed that there was "data from deletion studies that identified two domains as critical to activity Y, i.e., a binding domain and a catalytic domain". It also assumed that the applicants disclosed limiting mutations within these domains to conservative substitutions (per the aforementioned exchange groups), and permitting non-conservative substitutions outside those domains. Thus, "conservative substitutions" teachings were deemed relevant to an "activity" claim if coupled with some sort of "active site" teaching. The WDTM made it clear that it was not necessary that all of the suggested mutations in fact result in an active mutant, just that the art would expect that "many" of them would (see paragraph bridging pp. 41-42).

Here, our SEQ ID NO:2 is not the only known multisubstrate dNK and we have made specific comparisons of it to D. melanogaster, B. mori and A. gambiae dNKs (Fig. 1). The 3D structure of the D. melanogaster dNK is known (page 5, lines 36-327) and we have specifically identified the residues "closest to substrates", i.e., the binding residues (line 38). Consistently, these are residues conserved among the four dNKs of Fig. 1. Hence, under the standard of WDTM Exs. 10 and 11, claim 1 satisfies WD.

2.3. Claim 65 defines suitable polynucleotides on the basis of hybridization under medium stringent conditions. Such conditions are defined as requiring hybridization with 2xSSC, 0.5% SDS at a temperature of 60°C, whereas 65°C is medium/high, 70°C is high, and 75°C is very high. See page 10, lines 20-37.

The most relevant discussion in the WDTM pertains to Example 6, model claim 3: "An isolated nucleic acid that encodes a protein that binds to the NDG receptor and stimulates tyrosine kinase activity, wherein the nucleic acid hybridizes under highly stringent conditions to the complement of the sequence set fort

in SEQ ID NO:1". The PTO assumed that highly stringent conditions were defined so that the final wash was with 0.2 SSC and 0.1% SDS at 65°C. This is more stringent than our "medium/high" conditions, which has the same temperature but a higher salt concentration.

The PTO conceded that "the disclosure of SEQ ID NO:1 combined with the knowledge of the art concerning hybridization would put one in possession of the genus of nucleic acids that would hybridize under stringent conditions to SEQ ID NO:1". The problem was that the WDTM example assumed that the specification failed to disclose any art-recognized correlation between the disclosed function and structure. Hence, it held that WD was lacking.

However, we have amended claim 65 so as to eliminate reference to kinase activity. Hence, the sole issue is whether recitation of medium (rather than high) stringency is acceptable.

In this regard, we note that the use of medium or even low stringency hybridization is routine in the art to isolate, e.g., a cDNA encoding a related protein in the same species, a

(1) a DNA encoding a related protein of the same species,
(2) a cDNA encoding the homologous protein of a related species, or
(3) a genomic DNA encoding the same protein as that encoded by the cDNA probe.

For the use of probes to identify homologous genes in other species, see, e.g., Schwinn, et al., J. Biol. Chem., 265:8183-89 (1990) (hamster 67-bp cDNA probe vs. human leukocyte genomic library; human 0.32kb DNA probe vs. bovine brain cDNA library, both with hybridization at 42°C in 6xSSC); Jenkins et al., J. Biol. Chem., 265:19624-31 (1990) (Chicken 770-bp cDNA probe vs. human genomic libraries; hybridization at 40°C in 50% formamide and 5xSSC); Murata et al., J. Exp. Med., 175:341-51 (1992) (1.2-kb mouse cDNA probe v. human eosinophil cDNA library; hybridization at 65°C in 6xSSC); Guyer et al., J. Biol. Chem.,

265:17307-17 (1990) (2.95-kb human genomic DNA probe vs. porcine genomic DNA library; hybridization at 42°C in 5xSSC). The conditions set forth in these articles may each be considered suitable for the purpose of isolating homologous genes, and they are all less stringent than medium stringency as defined in our specification.

Hence, we respectfully assert that amended claim 65 satisfies WD.

In like manner, claim 66 recites "medium/high stringency". Even if 65 is found not to satisfy WD, 66 recites a significantly higher level of stringency, and it should be considered whether it satisfies WD. If the Examiner so holds, Applicants would be agreeable to an Examiner's Amendment to suitably revise claims 65 (and 6).

Claims 67 and 68, reciting "high" and "very high stringency", respectively, were not rejected on WD grounds.

3. Enablement (OA pp. 7-14)

Claims 1-3, 6-7, 9, 16, 19, 20, 22, 55, 59-63, 65, 66 and 69 stand rejected for insufficient enablement, the examiner conceding enablement of a polynucleotide encoding the polypeptide of SEQ ID NO:2.

Note that claims 7 (85% NT identity to SID1) and 69 (90% NT identity to SID1) are rejected for insufficient enablement even though they were not rejected for insufficient WD.

The Examiner defines genera (a)-(m), which differs as follows from those defined for purpose of the WD analysis:

(a) also mentions 85% identity to SID2; we aren't sure which claim the examiner was thinking of as claim 7 recites identity of the polynucleotide to SID1; and

(m) this corresponds to claim 69.

While the issue for written description was whether the completely identified species SID2 was "representative" of the claimed genera, and the issue for enablement purposes is whether it would require undue experimentation to identify operative

species within the claimed genera, on a practical level these are closely intertwined.

A species is "representative" of the claimed genus if the art would expect most of the untested members of the genus to have properties similar to that of the lead species. Such an expectation would be equally relevant in an enablement context as it would suggest that it would not require undue experimentation to identify additional operative species and that the frequency of inoperative species would be low.

However, in enablement analysis, it is not necessary that such a priori expectation exist. If screening of members of the genus is easy, and so the experimentation required isn't "undue", a high frequency of failures might be tolerated. See Ex Parte Chen, 61 SPQ2d 1025 (BPAI 2000) (success rate of integration of transgene 1%; "the number emphasized by the Examiner would reasonably appear to reflect the need for a repetitive procedure, rather than undue experimentation"). That is particular true if the claim explicitly excludes inoperative species, see Ex parte Mark, 12 USPQ2d 1904 (BPAI 1989).

(1) breadth of claims

The claims are limited to species encoding kinases highly similar (at least 85% identical) to the admittedly enabled SID2 (claim 1) or which hybridize at medium stringency to SID1 (claim 65). Claims reciting just 80% identity, and supported by a single example, were held to be enabled in Ex parte Kubin, 83 USPQ2d 140 (BPAI 2007). The number of species putatively covered by the claim is not particularly relevant, see In re Angstadt, 190 USPQ 214, 218 (CCPA 1976).

(3) state of the prior art

Three insect dNKs were already known in the art, see page 1, lines 23-30.

(4) relative skill of those in the art

The skill of workers in the molecular biology art is considered to be high, e.g., post-doc level.

(5) predictability or unpredictability of the art

While the effect of a mutation is not completely predictable, the specification provides guidance, see point (6).

(6) the amount of direction or guidance presented

This was already analyzed in the WD context; applicants identify conserved, semi-conserved and non-conserved regions, conservative substitution exchange groups, and active truncation mutants of DM-dNK.

(7) presence or absence of working examples

See examples; SEQ ID NO:2 was made and shown to be active.

(8) quantity of experimentation necessary

Molecular biology is not a "fledgling art". We have had over two decades of experience in mutating proteins.

If the skilled worker confines mutation to, e.g., the non-conserved residues of AE-dNK, then there are, according to Fig. 1, only 20 such residues. There are another 13 residues which are identified as semi-conserved. Thus, consideration of the teachings of page 7, lines 9-13 by itself reduces the number of mutation sites from 248 (length of SID 2) to 33 or even 20.

The general tolerance of these positions can quickly be determined by alanine-scanning mutagenesis, either as a series of fewer than 248 separately synthesized single substitution mutants, or in one fell swoop by combinatorial Ala-scanning mutagenesis. The Ala-scan could if desired be limited to the earmarked 33 or 20 positions. The skilled worker could choose not to further mutate an Ala-intolerant position.

As to the nature of the replacement residue, if the skilled worker is cautious and limits early experimentation to the conservative substitution mutants contemplated by page 9, group

(i) is eight amino acids, (ii) is six, (iii) is three, and (iv) is two. The extent to which this reduces the experimentation is dependent on amino acid composition, but if all amino acids were equiprobable, the reduction would be by about a factor of four ((20x19)/((8x7)+(6x5)+(3x2)+(2x1)).

Each mutant tested would of course provide information which would guide further experimentation.

4. Definiteness (OA pp. 14-17)

Claims 1-7, 9, 16, 19, 20, 22 and 55-72 stand rejected as indefinite.

4.1. The Examiner questions the phrase "a polynucleotide derived from a yellow fever mosquito" [sic]. The actual claim language was "polynucleotide encoding a mosquito deoxyribonucleoside kinase derived from a yellow fever mosquito".

In any event, we have deleted the questioned language.

4.2. The Examiner questions the Markush group formulation of claim 1 since "b" (the complement of "a") would not encode a dNK. We agree, and have suitably amended claim 1.

4.3. Claim paragraph 1(b) clearly means the complement to the full length of (a), whether that may be. Since (a) must encode a kinase having at least 85% identity to SID2 determined over the entire length of SID2, it follows that the length of (b) must be at least 85% the length of the complementary strand of SID2, and thus would exclude short probes and primers.

4.4. We have clarified claims 2 and 3 by introducing the term "thymidine kinase", as already implied by the "-TK1" in these claims.

4.5. We have amended claim 6 to require medium stringency conditions (cp. claim 65).

4.6. Claims 59 and 61 recite the terms "non-conserved" and "semi-conserved" as identified in Figure 1. A residue of SID 2 (Ae-dNK) is considered "non-conserved" if it appears as ordinary unshaded black-on-white text in Fig. 1. It is considered "conserved" if it appears in white-on-black text in Fig. 1. It

is considered "semi-conserved if it appears in shaded, black-on-white text in Fig. 1. This is all consistent with page 5, lines 34-36 and page 7, lines 8-13.

We have placed lists of (1) conserved residues and (2) semi-conserved residues into claims 59 and 61, and defined non-conserved residues as those neither (1) nor (2). A residue designation such as "M1" means that in SEQ ID NO:2 (Ae-dNK of Fig. 1), the methionine appears in position 1.

4.7. Claims 60 and 62 recite "an amino acid which appears at corresponding aligned position in another insect kinase".

As the examiner noted at page 5, second-to-last line, there are a "vast array of insects". As their kinases are isolated and sequenced (presumably by means similar to those used to isolate and sequence the kinases of Fig. 1, or other known kinases), they can be aligned with SEQ ID NO:2 and/or other Fig. 1 kinases by a single (BLASTP, p. 7; ALIGN p. 8) or multiple (e.g., Clustal W) sequence alignment program, whereupon corresponding aligned positions become available.

Imagine for a moment that Bombyx mori dNK sequence was not in Fig. 1. When it became available, it could be aligned with Ae-dNK, and that alignment was the one depicted in Fig. 1, then it would become apparent that for example Ae-dNK G14 could be replaced with Bm-dNK's N, or K15 with V, and so forth.

Claims of a similar nature have been granted in, e.g., Kopchick USP 5,958,879, claim 1 (the first and second reference vertebrate growth hormones are not limited to vertebrate GHs which had been sequenced at the time of filing).

5. Prior Art Issues (OA pp. 17-18)

5.1. The Examiner asserts that claims 1, 3, 6, 9, 16, 19, 20, 55 and 59-63 are anticipated by Knecht et al., who allegedly discloses a polynucleotide encoding a mosquito dNK 81.4% identical with our SID 2. We addressed this issue in section 1.1 (a posteriori lack of unity), and anticipation of claim 1, and all claims dependent thereon, is vitiated by the amendment of

claim 1 to recite at least 85% identity.

5.2. Claim 56 (reciting at least 90% identity) was not rejected over the prior art. Neither was claim 65, reciting hybridization under medium stringency conditions.

Respectfully submitted,

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